REMARKS

Claims 1-34 are pending, claims 9-15 and 29-34 are withdrawn from consideration, and claims 1 and 7 have been amended. No new matter has been added by virtue of these amendments and additions. The claim amendments and additions are supported by the specification and the originally-filed claims. In particular, support for the amendments to claim 1 can be found in the specification, for example, on page 10, second paragraph.

Amendment and cancellation should in no way be construed as an acquiescence to any of the Examiner's rejections. The amendments to, or cancellation of, the claims are being made solely to expedite prosecution of the present application and do not, and are not intended to, narrow the claims in any way. Applicants reserve the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

Rejection of Claims 1-8 and 16-28 under 35 U.S.C. § 112, Second Paragraph

Claims 1-8 and 16-28 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants respectfully traverse the rejection.

Claim 1 was rejected as allegedly indefinite "because it is unclear if the two or more columns having the protein ligand in varying concentrations immobilized in a matrix are in a sequence series one beside each other or if the columns are aligned in a series such that the varying concentrations are creating a gradient." Claim 1 has been amended and the amendments are believed to obviate the rejection. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Claim 7 was rejected as allegedly indefinite "because of the use of an acronym: MALDI-TOF." Claim 7 has been amended and the amendments are believed to obviate the rejection. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Rejection of Claims 1-6, 8, 16, 18-21, 23, 24, 27 and 28 under 35 U.S.C. § 103(a)

Claims 1-6, 8, 16, 18- 1, 23, 24, 27 and 28 were rejected under 35 U.S.C. §103(a) as being unpatentable over Patterson et al. (Electrophoresis 17: 877-891 (1996)) in view of Formosa et al. (Methods in Enzymology 208: 24-45 (1991)). The rejection is respectfully traversed.

Patterson et al. is relied on for disclosing affinity chromatography whereby the eluted proteins are subjected to gel electrophoresis and mass spectrometry. However, as stated in the Office Action, Patterson et al. fails to teach "two or more columns having a protein ligand in varying concentrations immobilized to a matrix" (Office Action at 4). Formosa et al. is relied on for disclosing "the use of multiple microaffinity columns having a ligand immobilized to a matrix in varying concentrations" (Office Action at 4). The Office Action states:

> It would have been obvious to one of ordinary skill in the art to incorporate multiple affinity columns having a protein ligand in varying concentrations immobilized to a matrix such as taught by Formosa et al into the method of Patterson et al because Formosa et al show that the use of such affinity columns allows for the dissociation constants of the protein-protein interactions to be estimated and also provides the advantage to screen for the effects of a variety of conditions on the binding of proteins from extracts.*** (Office Action at 4)

The claim amendments made herein are believed to obviate the rejection. In particular, the claims are directed, at least in part, to a method for identifying an interacting protein using affinity chromatography on two or more columns having varying concentrations of an immobilized protein ligand and wherein the amount of said interacting protein eluting from the columns varies proportionately with the concentration of immobilized protein ligand.

As stated in the Office Action, Patterson et al. fails to teach the use of two or more columns having a protein ligand in varying concentrations. Formosa et al. fails to cure the deficiencies of Patterson et al. In particular, Formosa et al. fails to teach or suggest a method for the identification of an interacting protein using two or more columns with varying ligand concentrations. In fact, Formosa et al. would appear to teach away from the use of two or more columns with varying ligand concentration as a method for identifying an interacting protein. Formosa et al. discloses that:

> Advantage can be taken of microcolumns to screen for the effects of a variety of conditions on the binding of proteins from extracts. An

> important variable to consider is the concentration of the immobilized protein ligand. There is often ***a critical optimum concentration that must be identified***: concentrations of the ligand that are ***too low*** do not retain enough protein from the extract, while concentrations that are ***too high*** can, with highly charged proteins, create ion exchangers that bind hundreds of proteins nonspecifically. An example of a ligand concentration curve using RNA polymerase II columns and mammalian whole-cell extract is shown in Figure 1A. (Formosa et al. at 35)(emphasis added)

Formosa et al. teaches that identification of an optimum concentration of ligand is essential. Furthermore, Formosa et al. teaches that concentrations of ligand that deviate from the optimum concentration may have negative effects. For example, ligand concentrations that are to low may result in failure to obtain adequate amounts of the interacting protein and ligand concentrations that are too high may result in significant nonspecific binding. Accordingly, one of ordinary skill in the art would not be motivated to ***isolate and/or identify an interacting protein*** using two or more columns with varying concentrations of ligand based on the teachings of Formosa et al. since Formosa et al. teaches that an optimal ligand concentration is essential for such purposes and that ligand concentrations which are too high or too low should not be used.

In sum, Formosa et al. merely teaches that the use of two or more columns with varying ligand concentrations would be useful for determining a single optimal ligand concentration for carrying out an affinity chromatography experiment. Formosa et al. fails to teach or suggest that two or more columns with varying ligand concentrations would be useful for carrying out affinity chromatography experiments in order to identify an interacting protein.

Formosa et al. also disclose that:

> The ***dissociation constants*** of the protein-protein interactions can be estimated from the results of microcolumn affinity chromatography experiments in which the ligand concentration is varied (Fig. 1A, for example). These columns are loaded with 3-10 column volume samples and washed with 10 column volumes of loading buffer before eluting the specifically bound proteins with salt or chaotropic agents. Proteins that remain bound after this extended washing must have dissociation constants at least 10-fold lower than the concentration of ligand coupled to the column. Therefore, ***the K_d of the interaction should be about one-twentieth of the lowest concentration of ligand that fully retains the binding protein***, assuming that all of the ligand is available for binding. (Formosa et al. at page 37)(emphasis added)

Formosa et al. teaches that the ***dissociation constant*** of a protein-protein interaction can be determined in experiments using affinity chromatography with varied ligand concentrations. The use of the varied ligand concentrations permits the identification of ***the lowest concentration*** of ligand which fully retains the binding protein. Once this lowest ligand concentration has been identified, the K_d of the interacting protein may be estimated to be about one twentieth of this concentration. Accordingly, Formosa et al. does not teach or suggest that the use of two or more columns with varying ligand concentrations would be useful for ***isolation and/or identification of an interacting protein***. Rather, Formosa et al. merely teaches that the use of multiple ligand concentrations would be useful to identify the lowest concentration of ligand that fully retains the binding protein so that a K_d may be estimated.

Additionally, Formosa et al. fails to explicitly or implicitly disclose the currently claimed embodiment. In particular, the claims are directed, at least in part, to a method for identification of an interacting protein using affinity chromatography on two or more columns having varying concentrations of an immobilized protein ligand and wherein the amount of said interacting protein eluting from the columns varies proportionately with the concentration of immobilized protein ligand. As stated above, Formosa et al. merely teaches that the use of two or more columns with varying ligand concentration may be useful to determine an optimal ligand concentration for affinity chromatography or for determining the lowest concentration of ligand that fully retains the binding protein so that a K_d may be estimated. Formosa et al. does not teach or suggest that the use of two or more columns with varying ligand concentrations would aid in the isolation and/or identification of an interacting protein. Nor does Formosa et al. teach or suggest that the amount of said interacting protein eluting from the columns varies proportionately with the concentration of immobilized protein ligand. In particular, Formosa et al. show the results of a ligand concentration curve experiment using RNA polymerase II immobilized on the columns in Figure 1A on page 36. Figure 1A shows the components eluted off the columns which were separated by SDS-PAGE and indicates three interacting proteins with arrows (RAP72, RAP38 and RAP30). The amount of ligand immobilized in each lane is shown at the bottom of the figure. Based on this figure, it would not be obvious to one of ordinary skill in the art that the amount of said interacting protein eluting from the columns varies proportionately with the concentration of immobilized protein ligand. In particular, the intensity of the RAP38 bands appear to have little correlation with the amount of ligand

immobilized on the column. For example, lanes d and h both have 10 μg of ligand immobilized on the column, but the amount of RAP38 eluted from the column appears to vary significantly between the two lanes. Further, lanes f (6 μg of immobilized protein) and lane h (10 μg of immobilized protein) would appear to show the same amount of eluted RAP38 even though the concentration of ligand immobilized on the column varies by almost two-fold. Accordingly, Formosa et al. fails to teach or suggest a method for identification of an interacting protein using affinity chromatography on two or more columns having varying concentrations of an immobilized protein ligand and wherein the amount of said interacting protein eluting from the columns varies proportionately with the concentration of immobilized protein ligand.

Applicants respectfully submit that "[t]o establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art." MPEP §2143.03 citing *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). Neither Patterson et al. nor Formosa et al., either alone or in combination, teach or suggest each and every element of the present invention. Accordingly, the cited references, either alone or in combination, fail to teach or suggest the currently claimed embodiments. Reconsideration and withdrawal of the rejection is respectfully requested.

Rejection of Claims 7, 17, 22, 25 and 26 under 35 U.S.C. § 103(a)

Claims 7, 17, 22, 25 and 26 were rejected under 35 U.S.C. §103(a) as being unpatentable over Patterson et al. in view of Formosa et al. as applied to claims 1-6, 8, 16, 18-21, 23, 24, 27 and 28 above, and further in view of Vestal et al (US 6,281,493).

Patterson et al. and Formosa et al. are relied upon for the teachings as described above. However, as stated in the Office Action, Patterson et al. and Formosa et al. fail to teach MALDI-TOF mass spectrometry. Vestal et al. is relied upon for disclosing the use of MALDI-TOF for measuring the mass-to-charge ratio of a sample molecule. In particular, the Office Action states that:

> It would have been obvious to one of ordinary skill in the art to incorporate the use of MALDI-TOF mass spectrometry as taught by Vestal et al into the modified method of Patterson et al because Vestal et al teaches that TOF mass spectrometers are advantageous because they are relatively simple, inexpensive instruments with virtually unlimited mass-to-charge range. Vestal et

> al also disclose that TOF mass spectrometers have potentially higher sensitivity than scanning instruments because they can record all the ions generated from each ionization event. (Office Action at 6)

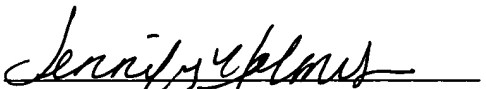
As discussed above, Patterson et al. in view of Formosa et al. fail to teach or suggest the currently claimed embodiment. Vestal et al. fail to make up for the deficiencies of Patterson et al. and Formosa et al. In particular, Vestal et al. fails to teach or suggest a method for identification of an interacting protein using affinity chromatography on two or more columns having varying concentrations of an immobilized protein ligand and wherein the amount of said interacting protein eluting from the columns varies proportionately with the concentration of immobilized protein ligand. Accordingly, the cited references, either alone or in combination, fail to teach or suggest the currently claimed embodiments. Reconsideration and withdrawal of the rejection is respectfully requested.

Applicants believe that the claim amendments and remarks made herein fully address all issues raised in the Office Action. Silence with regard to any of the Examiner's rejections is not an acquiescence to such rejections. Specifically, silence with regard to Examiner's rejection of a dependent claim, when such claim depends from an independent claim that Applicant considers allowable for reasons provided herein, is not an acquiescence to such rejection of the dependent claim(s), but rather a recognition by Applicant that such previously lodged rejection is moot based on Applicant remarks and/or amendments relative to the independent claim (that Applicant considers allowable) from which the dependent claim(s) depends.

CONCLUSION

Applicants consider the Response herein to be fully responsive to the referenced Office Action. Based on the above Remarks and Amendment, it is respectfully submitted that this application is in condition for allowance. Accordingly, allowance of the pending claims is requested. If a telephone conversation with Applicants' Agent would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 832-1000.

Respectfully submitted,

By: 
Jennifer K. Holmes, Ph.D.
Reg. No. 46,778
Agent for Applicants

Patent Group
FOLEY HOAG LLP
155 Seaport Blvd.
Boston, MA 02210-2600

Telephone: (617) 832-1000
Facsimile: (617) 832-7000

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